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Studies on *Microthyriaceae*: some excluded generaHAIXIA WU^{1*}, YANMEI LI¹, HANG CHEN¹ & KEVIN D. HYDE^{1, 2, 3}

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Abstract — The genera *Asteronia*, *Dictyoasterina*, *Helminthopeltis*, and *Hidakaea* are presently included in the *Microthyriaceae*. We have examined the types, and their characters do not agree with this familial placement. We redescribe these four poorly known genera and suggest a more appropriate placement of these genera. *Asteronia* produces subglobose ascomata that develop on dark mycelium with copious hyphopodia on the host surface and should be placed in the *Asterinaceae* or *Meliolaceae*. *Dictyoasterina* has a black mycelial net, superficial hyphae with lateral hyphopodia, and globose ascomata with an ostiole and can be placed in *Asterinaceae*. The monotypic *Helminthopeltis*, characterized by elongated longitudinally cleft ascomata and filiform hyaline one-celled ascospores, should be placed in *Rhytismataceae*; *H. almeidaeana* is probably a synonym of a species in *Lophodermium*. *Hidakaea* has brown ascomata and unitunicate asci and should be considered in *Hypocreales*.

Key words — *Asteronia sweetiae*, *Dictyoasterina conopharyngiae*, *Helminthopeltis almeidaeana*, *Hidakaea tumidula*, taxonomy

Introduction

We are conducting studies on the *Dothideomycetes* in order to provide a natural classification (Zhang et al. 2008, 2009). As part of this work we are restudying the type species of the 49 genera placed in the *Microthyriaceae*, a poorly known but interesting family within the *Dothideomycetes* (Lumbsch & Huhndorf 2007). The important morphological characters of *Microthyriaceae* are superficial, flattened, dimidiate ascomata, the cells of upper wall of which are organized in

a radiating pattern, while a lower peridium is generally lacking. Members have a central ostiolar opening, cylindrical or broadly clavate to saccate, bitunicate and fissitunicate asci and ascospores that are hyaline or brown (Ryan 1924, 1926; Kirk et al. 2008). We have thus far examined several taxa within *Microthyriaceae* and in this paper report on four poorly known genera and their types: *Asteronia* (Hennings 1895) represented by *Asteronia sweetiae*, *Dictyoasterina* (Hansford 1947) represented by *Dictyoasterina conopharyngiae*, *Helminthopeltis* (Sousa da Câmara 1950) represented by *Helminthopeltis almeidaeana*, and *Hidakaea* (Hino & Katumoto 1955) represented by *Hidakaea tumidula*. Full descriptions of these taxa and suggestion for their taxonomic placement are provided.

Materials and methods

Type specimens of *Asteronia sweetiae*, *Dictyoasterina conopharyngiae*, *Helminthopeltis almeidaeana*, and *Hidakaea tumidula* were obtained from K, IMI, LISE and YAM, respectively. Ascomata were rehydrated in 3% KOH prior to examination and sectioning. Specimens were examined under a stereo microscope (Leica MZ16A) and fine forceps were used to remove one or two ascomata, which were mounted in water, Melzer's, Congo red, or cotton blue reagents. Observations and photographs were made under the light microscopes (Nikon E800 and Leica DM3000). For some hyaline structures differential interference contrast microscopy was used.

Hand sections were cut with a sharp razor blade and thin (8 μm) sections were cut using a Leica CM1100 freezing microtome. The sections were transferred to a drop of water or a drop of cotton blue for examination and photography.

Taxonomy

Asteronia (Sacc.) Henn., Hedwigia 34: 104, 1895.

FIG. 1A–I

TYPE SPECIES: *Asteronia sweetiae* Henn., Hedwigia 34: 104, 1895.

$\equiv$ *Parodiopsis sweetiae* (Henn.) G. Arnaud, Annales des Épiphyties. 7: 53, 1921.

Colonies forming darkened regions on the underside of leaves, resembling a “sooty mold” to the unaided eye. Ascomata 130–180 μm high $\times$ 110–140 μm diam, gregarious, or some scattered, superficial, subglobose to globose, subcoriaceous, brown to black–brown, with an indistinct ostiole (FIGS 1A, B, C). Peridium 17–24 μm wide, one layered, composed of black-brown isodiametric cells of *textura angularis* (FIGS 1D, E). Hamathecium not apparent. Asci 60–93 $\times$ 20–27 μm (mean = 72 $\times$ 22.8 μm), 8-spored, bitunicate, oblong to broadly cylindrical, with a short pedicel, 8–9 μm long, 6.5–7.5 μm wide, apically rounded with an ocular chamber (FIGS 1F, H). Ascospores 33–46 $\times$ 4–6.5 μm (mean = 40.8 $\times$ 5.4 μm), tri-seriate to multiseriate, guttulate, thin-walled, straight or slightly curved, fusoid-ellipsoidal, widest in the middle part of the apical cell, with broadly rounded apex and tapering to a narrowly rounded base, hyaline, 1-septate, septum nearly central but nearer to apex, wall rough (FIGS 1G, I).



FIG. 1. *Asteronia sweetiae* (from holotype). A. Colonies of fungus on underside of host leaves. B. Appearance of ascomata on the host surface. C. Squash mount of ascoma. D, E. Section of ascomata. Note peridium. F, H. Asci Note the pedicel and ocular chamber. G, I. Ascospores. Scale bars: B = 200µm, C = 20 µm, D–I = 10 µm.

SPECIMEN EXAMINED: BRAZIL, Estado de Minas Gerais; on leaves of *Sweetia* sp. (*Fabaceae*), June 1892, E. Ulé (1968) (K (M) 159800, holotype).

Asteronia was described as a monotypic genus represented by *Asteronia sweetiae*. Subsequently, Hennings (1908) added *A. lauraceae* Henn. to the

genus. Two other names listed under *Asteronia* by Index Fungorum are errors for *Asterina appendiculosa* (Mont. & Berk.) Mont. and *Asterina erysiphoides* Kalchbr. & Cooke, species that were not included in *Asteronia* by Hennings (1895, 1908) and which have not been accepted or recombined by subsequent authors. Saccardo & Sydow (1899: 693) described *A. sweetiae* as parasitic with mycelium on the lower surface of leaves, ascomata that are subglobose and gregarious, and asci that are bitunicate with 8 spores. *Asteronia* is currently placed in the family *Microthyriaceae* (Lumbsch & Huhndorf 2007). However, the familial classification of *Asteronia* has been long confused. Hennings (1895) placed *Asteronia* in *Perisporiaceae* (= *Meliolaceae*), Saccardo & Sydow (1899) in *Microthyriaceae* subfam. *Asterinoideae*, Hennings (1908) in *Microthyriaceae*, and Arnaud (1921) in *Parodiellinaceae* (= *Parodiopsidaceae*).

Asteronia sweetiae has globose, ostiolate ascomata that form on black mycelium, while asci form from the base of ascomata. These characters and lack of flattened thyriothecia indicate that this genus is not well placed in *Microthyriaceae*. A more suitable family is probably *Asterinaceae* or *Meliolaceae*. However, there are no sequence data for *Asteronia* in GenBank and fresh collections are needed in order to establish the phylogenetic relationship of this genus.

Dictyoasterina Hansf., Proceedings of the Linnean Society of London
159: 39, 1947.

FIG 2A–K

TYPE SPECIES: *Dictyoasterina conopharyngiae* Hansf., Proceedings of the Linnean Society of London 159: 39, 1947.

Epiphytic on the upper surface of leaves, appearing as blackened areas, which are rounded and shiny, scattered over the leaf, with superficial hyphae present, forming a black mycelia net (FIGS 2A, B). Superficial hyphae, black to brownish, parallel and anastomosing, with sparse, lateral hyphopodia, hyphopodia nearly circular, black-brown, 20.5–28 μm (mean = 26 μm) (FIG 2D). Ascomata 38–100 μm high, 90–220 μm diam, superficial, gregarious, roughly globose, black, subcoriaceous or less carbonaceous, with a central ostiole (FIG 2C). Peridium 6–18 μm wide comprised of three layers of cells, black-brown to pale brown, outer layer of black brown cells compressed, inner layer of isodiametric cells of textura epidermoidea (FIGS 2E, G). Hamathecium of dense, long pseudoparaphyses, 50–61 $\times$ 1.5–3 μm (mean = 55 $\times$ 2.4 μm) unbranched (FIGS 2F, I). Asci 32–47.5 $\times$ 8–13 μm (mean = 35.9 $\times$ 9.9 μm), 8-spored, bitunicate, fissitunicate, clavate to short cylindrical, with a short knob-like pedicel 1.7–3.2 μm , and inconspicuous apical structure (FIG 2H). Ascospores 9.5–15 $\times$ 3–6 μm (mean = 11.8 $\times$ 4.4 μm), biseriate to overlapping triseriate, oblong-ellipsoid to obovate, 1-septate, hyaline, strongly constricted at the septum, upper cell oval, wider and shorter than the cone-shaped lower cell, guttulate, smooth-walled (FIGS 2J, K).



FIG. 2. *Dictyoasterina conopharyngiae* (from holotype). A. Appearance of fungus on surface of leaf. B. Appearance of colony and ascomata on the host surface. C. Squash mount of ascoma. D. Hyphopodia. E. Section of ascoma. Note the peridium which comprises several layers of cells. F. Asci and paraphyses. G. Peridium. H. Asci mounted in Melzer's reagent. I. Paraphyses. J, K. Ascospores becoming red when mounted in Congo red and yellow to light brown in Melzer's reagent. Scale bars: B = 200µm, C = 20 µm, D–K = 10 µm.

SPECIMEN EXAMINED: UGANDA, Entebbe, on leaf of *Conopharyngia holstii* (*Apocynaceae*), December 1945, C.G. Hansford, (IMI5298, holotype).

Dictyoasterina was described as a monotypic genus represented by *D. conopharyngiae* and was placed into the family *Asterinaceae* (Hansford 1947). In the latest Myconet, *Dictyoasterina* is removed into the family *Microthyriaceae* (Lumbsch & Huhndorf 2007).

Dictyoasterina conopharyngiae has superficial mycelium and clearly ostiolate, globose ascomata; these morphological characters make it distinct



FIG.3. *Helminthopeltis almeidaeana* (from holotype). A. Appearance of ascomata on the host surface. B, C. Squash mount of ascoma. D–F, L. Section of ascomata. Note peridium. G, I, K, M. Unitunicate asci. H, J. Ascospores. Scale bars: A = 500 μm , B = 20 μm , C–M = 10 μm .

from *Microthyriaceae*. We suggest that *Dictyoasterina* be excluded from *Microthyriaceae* and transferred to a more suitable family, such as the

Asterinaceae, although the presence of pseudoparaphyses should be considered. As there are no sequences for this genus in GenBank, fresh collections and DNA sequence analyses are still needed to establish taxonomic placement.

Helminthopeltis Sousa da Câmara, *Agronomia Lusitana* 12: 102, 1950. FIG 3A–M

TYPE SPECIES: *Helminthopeltis almeidaeana* Sousa da Câmara, *Agronomia Lusitana* 12: 102, 1950.

Necrotrophic or biotrophic on the surface of leaves of conifers, forming black oval spots. Superficial mycelium absent. *Ascomata* 140–205 µm long × 75–110 µm wide, about 100–150 µm high, solitary or grouped in pairs, scattered, superficial, clypeate, oblong or elongated, with a longitudinal cleft-like opening, subcoriaceous, black to brown (FIGS 3A, B, C). Peridium 50–72 µm thick at the apex, 25–35 µm thick at base, two-layered, composed of hyaline pseudoparenchymatous cells and an inner layer of isodiametric cells of *textura angularis* (FIGS 3D–F, L). Hamathecium sparse or absent. Asci 60–117 × 10–18 µm (mean = 77 × 15.2 µm), at least 8-spored (or more than 8-spored), not fissitunicate, cylindrical to oblong, thin-walled, parallel arrangement (FIGS 3G, I, K, M). Ascospores 62.5–83 × 2–3.5 µm (mean = 70.9 × 2.8 µm), parallel seriate, broad filiform to wire-like, hyaline, aseptate, wall smooth (FIGS 3H, J).

SPECIMENS EXAMINED: PORTUGAL, Minho Serra do Gerez (Pico Borrageiro), on leaf of *Juniperus communis* (*Cupressaceae*), 3 July 1948, M. de Sousa da Câmara (LISE 50024, holotype).

Sousa da Câmara (1950) erected *Helminthopeltis* as a monotypic genus for *H. almeidaeana*, which is found only in Europe (Kirk et al. 2008). The 2007 Outline of *Ascomycota* (Lumbsch & Huhndorf 2007) places *Helminthopeltis* into *Microthyriaceae*. We suggest that *H. almeidaeana* is better placed in *Lophodermium* (*Rhytismataceae*) but do not transfer the species here as it has probably already been described in this large genus under another name.

Hidakaea I. Hino & Katum., *Bulletin of the Faculty of Agriculture, Yamaguchi University* 6: 38, 1955. FIG 4A–K 5A–D

TYPE SPECIES: *Hidakaea tumidula* I. Hino & Katum., *Bulletin of the Faculty of Agriculture, Yamaguchi University* 6: 38, 1955.

Saprobic or parasitic on stems of bamboo, causing black to brown spots. *Ascomata* 260–270 µm diam. 120–160 µm high, solitary or gregarious, flattened against the host surface, subglobose, in section hemispherical, brown, subcoriaceous but membranous at the base, smooth from above, unilocular, with a central ostiole (FIGS 4A, B). Ostiole 6.0–6.5 µm. Peridium 41–63 µm wide, brown-black at the sides, other parts light brown, in the sagittal section, comprising a few layers of cells, outer cells appear pseudoparenchymatous and cell wall very thin, on the base cells isodiametric, black-brown (FIGS 4C,

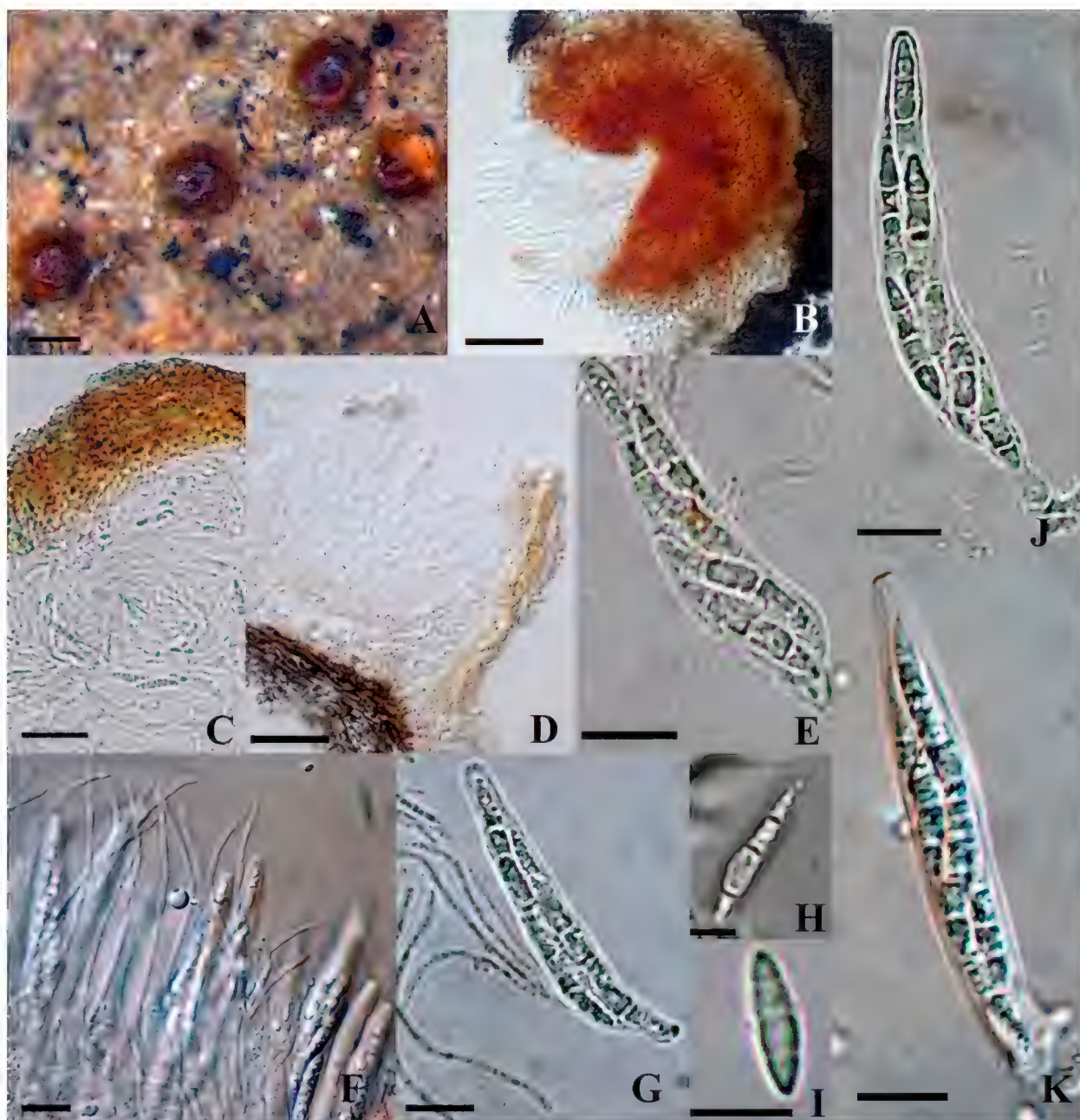


FIG. 4. *Hidakaea tumidula* (from holotype). A. Appearance of ascomata on the host surface. B. Squash mount of ascoma. C, D. Section of ascoma. Note the peridium. E, J, K. Asci. Note the pedicel and the apical ring structure. F, G. Paraphyses which are longer than asci. H, I. Ascospores. Note the three septa and inconspicuous sheath. Scale bars: A = 200 μm ; B, C, D = 20 μm ; E–G, J, K = 10 μm ; H, I = 5 μm .

D). Hamathecium comprising paraphyses, $70\text{--}105 \times 1\text{--}2 \mu\text{m}$, embedded in mucilage, and longer than asci (FIGS 4F, G). Asci $71\text{--}82 \times 7\text{--}10 \mu\text{m}$, 8-spored, unitunicate, cylindrical-clavate or oblong, with a short pedicel $6 \times 4 \mu\text{m}$, apically rounded with inconspicuous apical structure (FIGS 4E, J, K). Ascospores $16\text{--}20.5 \times 2.5\text{--}4 \mu\text{m}$, 2-seriate, fusiform, tapering gradually at one or both ends, or pointed, hyaline or pale, 3-septate, slightly constricted at septa, smooth-walled, some with sheath (FIGS 4H, I).

SPECIMEN EXAMINED: JAPAN, Sagami Province, Qoyama, on dead stems of *Pleiblastus vaginatus* (Poaceae, Bambusoideae), September 1952, Hino and Katumoto (YAM 20296, holotype).

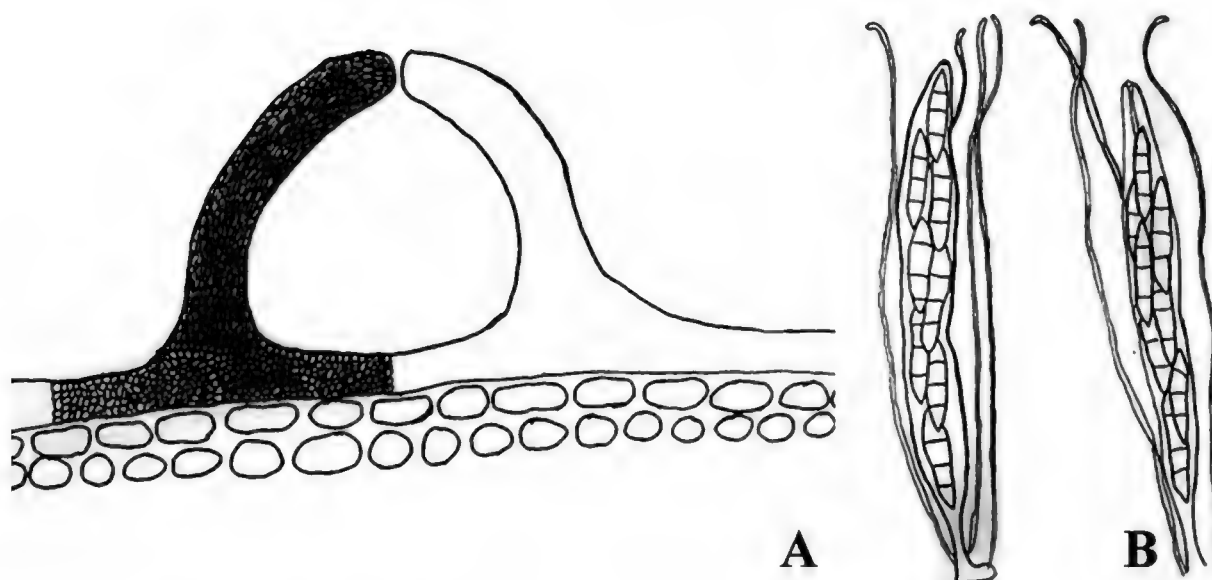


FIG.5. *Hidakaea tumidula* (line drawing from holotype). A. Ascoma. B. Asci.

Hino & Katumoto (1955) established *Hidakaea* as a monotypic genus with *H. tumidula* as the type species and suggested that it should be placed into *Microthyriaceae* based on ascomata with a scutate structure, superficial subglobose ostiolate ascomata, and bitunicate, clavate or cylindrical-clavate asci. The scutate structure and bitunicate asci described in the prologue were not observed in the holotype. The unitunicate ascus of *Hidakaea* does not belong in *Dothideales* and *Microthyriaceae*. The species should be placed the *Sordariomycetes* where it may have affinities with the *Chaetosphaeriales* or *Hypocreales*. The brown colour of the ascomata is typical of species in the *Hypocreales*, and the asci and ascospores are also characteristic of this order. We therefore suggest that *Hidakaea* belongs in *Hypocreales* incertae sedis.

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